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16. ABSTRACT

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Acute Toxicity of Thiocyanate to Trout

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Abstract

Acutely toxic effects of thiocyanate (SCN^-) were studied in brook trout *Salvelinus fontinalis* and rainbow trout *Salmo gairdneri* exposed to concentrations of up to 518 mg/L SCN^-. These fishes were capable of accumulating thiocyanate against its concentration gradient and at relatively rapid rates dependent upon the exposure SCN^- concentration, fish size, and anionic composition of the exposure water. Uptake of thiocyanate was inhibited by external chloride, was accompanied by a decline in the plasma Cl^- concentration, and, therefore, presumably involved substitution of SCN^- for Cl^- at the HCO_3^-/Cl^- exchange sites of the gill. Endogenous conversion of thiocyanate to cyanide was detected in exposed trout; the molar equilibrium ratio of SCN^- to HCN in blood was approximately 664:1. Determinations of 96-h median lethal concentrations were of limited value in assessing the toxicity of thiocyanate because of anomalous deaths of exposed fish. These deaths were characterized by convulsions, gasping, loss of equilibrium and buoyancy, flaring of the opercular, darkening of the skin epithelium and, within minutes, cessation of ventilation and extreme rigor. This "sudden death syndrome" (SDS) could be triggered by strenuous exercise, abrupt changes in photoperiod, and increased levels of spontaneous activity. The SDS may involve a direct effect of SCN^- on neuromuscular functioning. Prevalence of SDS was closely correlated with the plasma thiocyanate concentration: SDS occurred in half the fish when plasma concentrations reached approximately 250 mg/L SCN^-.

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Information on the toxicity of thiocyanate (SCN^-) to aquatic lifeforms is scant and contains many conflicting results (for reviews see Doudoroff 1976; Towill et al. 1978; Huiatt et al. 1983). Doudoroff (1976), for example, listed studies showing effect thresholds at experimental concentrations ranging from 29 to 5,000 mg/L SCN^-; he concluded that "thiocyanate is somewhat toxic." The APHA et al. (1980) consider thiocyanate toxicity to be unimportant unless subsequent chlorination, which would produce cyanogen chloride, is anticipated. The U.S. Environmental Protection Agency (USEPA 1980) does not include a criterion for thiocyanate. Further examination of the aquatic toxicity of thiocyanate is timely because of recent development of the SO_2–air process for cyanide removal from industrial effluents. This process selectively oxidizes cyanide and metal cyanide complexes, but has little effect on thiocyanate (Devuyst et al. 1982; Huiatt et al. 1983). Huiatt et al. (1983), for example, report that SCN^- concentrations in a base-metal mill effluent following SO_2–air treatment ranged from 168 to 680 mg/L, equivalent to approximately 80% of the feed SCN^- concentrations.

Thiocyanate enters the aquatic biosphere directly as SCN^- and indirectly as a product of the metabolism of cyanide. Endogenous conversion of cyanide to thiocyanate is the principal pathway for cyanide detoxification in many animals (for review see Vennesland et al. 1981). This does not mean, however, that thiocyanate is not toxic. Thiocyanate inhibits transport of halides in the thyroid (Wolff 1964), stomach (Davenport 1940; Davies 1951), cornea (Zadunaisky et al. 1971), and gills (Epstein et al. 1973; Epstein et al. 1975), amongst other sites, and inhibits such enzymes as carbonic anhydrase (Davenport 1940), succinic dehydrogenase (Solomon et al. 1973), and moieties of ATPase (Katz and Epstein 1971; Solomon et al. 1973). Thiocyanate has been used

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therapeutically in the treatment of hypertension in humans, occasionally with unpredictable and fatal consequences (Garvin 1939; Goodman and Gilman 1970). Toxic effects on humans are due to an action of SCN^- on the central nervous system and include irritability, nervousness, hallucinations, psychosis, mania, delirium, and convulsions (Barnett et al. 1951). There is some controversy as to whether or not the toxic effects of thiocyanate involve endogenous generation of cyanide (Vesey and Wilson 1978). Evidence of the conversion of inorganic and organic thiocyanates to cyanide has been found in mammals (Goldstein and Reiders 1951; Boxer and Rickards 1952a, 1952b; Pines and Crymble 1952). These conversions are reported to involve a red cell enzyme, thiocyanate oxidase (Goldstein and Reiders 1953), and the enzyme glutathione S-transferase (Habig et al. 1975). The physiological importance of such endogenously generated cyanide has yet to be ascertained. Recent work, however, has shown that antidotes for cyanide poisoning have no effect on the acute toxicity of thiocyanate to mammals (Smith 1973).

The present studies were undertaken at Fisheries Bioassay Laboratory (FBL) initially to determine the 96-h median lethal concentration (96-h LC50) of thiocyanate to trout. Studies with brook trout *Salvelinus fontinalis* were undertaken in 1981, and results of those studies were confirmed in general with rainbow trout *Salmo gairdneri* in 1982; the studies then were expanded to include examination of the physiological effects of thiocyanate exposure. These included examination of the rate of accumulation of thiocyanate in the fish blood plasma, rate of urinary excretion, and effects on blood ionic and acid–base status.

Methods

Acute Toxicity Studies

Four different types of acute toxicity tests were conducted: conventional 96-h toxicity tests both with and without controlled light conditions; conventional 96-h toxicity tests following which fish were stressed and additional mortalities recorded; 96-h toxicity tests during which fish were periodically stressed at 24-h intervals; and a test in which separate groups of fish were exposed to the same concentration of toxicant for the same time period and subsequently exposed to water without toxicant and then stressed, but the respite time period before stress differed for each group.

Brook trout (6–9 g) and rainbow trout (0.6–2.8 g) for these tests were obtained from the Fish Cultural Development Center (FCDC), U.S. Fish and Wildlife Service, Bozeman, Montana, and the Spring Creek Trout Hatchery, Lewistown, Montana. The fishes were held and tested at the FBL Bridger Laboratory, located at FCDC, in water obtained from a ground spring source (Table 1). Fishes were fed a hatchery production diet (Rangen, Incorporated, Buhl, Idaho) during rearing, but were not fed for 2 d prior to, or during the tests.

Acute (96-h) toxicity tests were conducted under continuous-flow conditions in either 60-L plastic tanks or 35-L glass tanks. Each test included a control and five toxicant concentrations; toxicant concentrations were maintained by proportional diluters modified from the design of Mount and Brungs (1967). Reagent grade potassium thiocyanate (KSCN) solution was used as the toxicant. Flow rates into the plastic tanks were 250 mL every 2–3 min and flows into the glass tanks were 500 mL every 2–3 min, providing a water replacement time of approximately 5 h in the plastic tanks and approximately 3 h in the glass tanks. Full toxicant concentration in each tank was effectively reached within 18 h.

Fish for a given test were distributed randomly among the tanks (9–15 fish per tank) and allowed 48 h to acclimate to the test environment without toxicant. Dead fish were counted and removed from each tank after 4, 8, 12, 48, and 96 h into each test. Tests were conducted under two conditions: at ambient photoperiod with fish visually exposed to normal laboratory traffic and activities, and at controlled photoperiods with fish isolated by curtains from normal laboratory activities. Controlled photoperiods were 24 h white light (220 lux), 12 h white light and 12 h darkness, and 24 h dim red light (0.2 lux). In controlled-photoperiod tests, spontaneous activity of the fish was recorded as the number of interactions between individuals over a 5- to 10-min period. Effects of stress on thiocyanate toxicity were assessed in tests in which fish were chased with a hand-held dip net and forced to swim vigorously for 5–15 s; care was taken not to touch the fish during this procedure.

Chemical analyses of the test solutions were performed one to three times in each tank during each test. Oxygen tensions were measured with

a dissolved-oxygen meter (Yellow Springs Instrument Company, model 54); temperature with a calibrated mercury thermometer; pH with a Beckman Phasar-I meter; thiocyanate, alkalinity, and hardness according to standard analytical procedures (USEPA 1974; APHA et al. 1980).

Physiological Studies

Six experiments were conducted to determine the physiological response of fish, including accumulation of SCN^- in the blood, under different test conditions. In three of these experiments, SCN^- concentrations were measured in blood taken from the caudal arteries of the fish. These tests were designed to determine the occurrence of "sudden death syndrome" (described later) versus blood plasma SCN^-, fish size versus rate of blood plasma SCN^- accumulation, and possible inhibition of SCN^- uptake by the presence of different halides in the test water. In three other experiments, blood (and urine) was sampled from indwelling cannulae in live fish. These tests were designed to determine possible changes over time in blood pH, CO_2, SCN^-, and Cl^-, the rate of urinary excretion of SCN^- versus blood plasma SCN^-, and the relationship over time among SCN^-, CN^-, and ammonia-nitrogen in blood plasma.

All of these experiments were conducted on rainbow trout (5–200 g), obtained from FCDC. The fish were held and tested at the FBL Cooley Laboratory, located on campus at Montana State University, in flowing, dechlorinated Bozeman city water (Table 1), and under a 12-h-light: 12-h-darkness photoperiod. Fish were fed the same diet as that given the fishes used for the acute toxicity studies; they were not fed for 2 d prior to, or during, the tests.

Indwelling cannulae were implanted in the dorsal aorta of fish (>100 g) under anaesthetic (MS-222 67 mg/L, buffered to pH 7.5 with $NaHCO_3$) as described by Smith and Bell (1964). A catheter was also implanted in the bladder via the urethra of some of the fish at this time. Fish were allowed at least 48 h to recover following surgery. This recovery period is more than adequate to allow the chemical characteristics of blood to return to preoperative conditions (Houston et al. 1971).

Cannulation of the dorsal aorta of fish smaller than 100 g was not possible in our hands. Blood samples from these fish were collected from severed caudal blood vessels after the fish were stunned by a blow to the head. Chemical characteristics of blood collected in this or any similar manner must be treated with caution because of the known effects of physical and anoxic stress on blood composition (for review see Pickering 1981). We conducted a preliminary study of this sampling technique and found it had no measurable effect on plasma thiocyanate concentration; in this preliminary study the plasma thiocyanate concentration of blood samples collected from the dorsal aorta of cannulated, unrestrained rainbow trout were compared with samples collected immediately thereafter by stunning the same fish and severing the caudal blood vessels.

Fish for a given test were transferred to tanks with continuous water flow and were allowed at least 48 h to acclimate to the test environment without toxicant. Some fish were then exposed to solutions of thiocyanate (reagent grade KSCN) delivered via micrometering pumps (Fluid Metering Incorporated, Oyster Bay, New York), while a control group was left undisturbed. In one set of experiments, several univalent anions (F^-, I^-, Br^-, Cl^-, $C_2H_3O_2^-$) were tested individually in equimolar concentrations with SCN^- to determine possible competitive inhibition of thiocyanate uptake.

TABLE 1.—*Chemical characteristics of dilution water used in thiocyanate studies.*

Chemical	Bridger Laboratory water, mg/L	Cooley Laboratory water, mg/L
Al	<0.02	<0.02
Ba	0.031	0.043
Be	<0.002	<0.002
B	<0.01	<0.01
Cd	<0.5	<0.5
Ca	46	19
Cl	<1	13
Cr	<0.1	<0.1
Cu	<0.002	<0.002
Pb	<0.3	<0.3
Mg	9.8	6.2
Mn	0.006	<0.002
Mo	<0.05	<0.05
Ni	<0.05	<0.05
P	<1	<1
K	0.48	1.3
Si	0.3	5.6
Na	0.6	2.6
Sr	<0.05	<0.05
Alkalinity (as $CaCO_3$)	172	81
Hardness (as $CaCO_3$)	201	81

Blood samples were taken either serially throughout an experiment or singly from each fish at the end of an experiment. Serial blood samples from aortic cannulae (0.35 mL per sample) were replaced with an equivalent volume of teleost saline (Wolf 1963). Haematocrit was determined on a portion of each whole blood sample; other measurements were made on plasma obtained by anaerobic centrifugation of blood samples. Determination of plasma pH was made anaerobically at the ambient water temperature with a pH–blood gas monitor (Radiometer PHM-74) and associated microelectrodes (type G297/G2). Total carbon dioxide content (sum of all CO_2 species present) of plasma was determined with a CO_2 electrode (Radiometer, type E5036) as described by Cameron (1971). Chloride, thiocyanate, cyanide, and total ammonia-nitrogen (NH_3-N) in body fluids were determined by methods of Ramsay et al. (1955), Pettigrew and Fell (1972), Rodkey and Collison (1977), and Seligson and Hirahara (1957).

Data Treatment and Analyses

The 96-h LC50 values and their 95% confidence intervals (95% CI) were calculated by the trimmed Spearman–Karber method (Hamilton et al. 1977). Standard treatment of data included analysis of variance and Duncan's multiple-range test. A 5% level of probability ($P \leq 0.05$) was adopted as the criterion of statistical significance. Data are reported as arithmetic means ± 1 SE.

Results

Acute Toxicity

In our initial conventional 96-h acute toxicity tests, the toxicity of thiocyanate to trout proved to be extremely variable. Although there was a general exposure–response correlation, these tests on brook trout indicated that some fish survived the 96-h test periods at thiocyanate concentrations an order of magnitude greater than concentrations that killed a high percentage of other fish (Table 2). Moreover, the death of fish frequently was sudden; fish that evidenced little or no sign of distress during scheduled observations were dead within minutes thereafter. These sudden deaths were characterized by tonic convulsions, loss of equilibrium and buoyancy, gasping, flaring of the operculae, darkening of the skin epithelium and, within minutes, cessation of ventilation and extreme rigor. We termed this occurrence "sudden death syndrome" (SDS).

To test the hypothesis that SDS was related to disturbance of the fish, two tests, one each on brook trout and on rainbow trout, were conducted with laboratory activities near the test area reduced to a necessary minimum. At the close of the standard 96-h test period, the fish were stressed with a dip net. Mortality counts taken 15 min later (brook trout) or 75 min later (rainbow trout) showed a dramatic increase over those taken at 96 h (Table 3) due to the occurrence of SDS.

Thiocyanate toxicity was influenced by the frequency with which fish were stressed. Two additional 96-h tests on brook trout were conducted during which the fish were stressed with a dip net after 24, 48, 72, and 95 h of exposure. Fish size and measured water chemistry variables for these tests were similar to those reported for brook trout in Table 3. The 96-h LC50 values for tests in which fish were stressed at 24-h intervals were 13.8 mg/L SCN^- (95% CI, 12.5–15.3) and 13.3 mg/L SCN^- (95% CI was not calculable by the method used), less than that of unstressed fish (>16.7 mg/L, Table 3) but greater than that of fish which were stressed only once after 96 h exposure (7–8 mg/L, Table 3).

The data are insufficient to conclude whether or not thiocyanate toxicity is influenced by photoperiod (Table 4). There would appear to be a trend toward greater toxicity as the duration of the white light period increased, but such a conclusion is tenuous. Photoperiod, however, was related directly to the level of spontaneous activity of the fish in each test situation. A three-fold increase in activity between fish under 24-h dim red light (mean activity 0.38 ± 0.13 interactions/min) and fish under 24-h white light (mean activity 1.16 ± 0.41 interactions/min) accompanied the approximate three-fold increase in thiocyanate toxicity between these two studies. At the end of the 96-h test periods, fish tested under continuous red light were briefly and abruptly exposed to white light, while fish tested under continuous white light were briefly and abruptly exposed to total darkness. Mortality counts taken 45 min later (96.75 h total exposure) indicated that startling fish in this way was sufficient to induce SDS and, hence, to increase the toxicity of thiocyanate (Table 4).

The persistence of SDS was tested by stressing (dip-net method) separate groups of juvenile

TABLE 2.—*Percent mortality of brook trout during 96-h acute toxicity tests with thiocyanate.*[a] *Data from four tests are combined and ranked in decreasing order of exposure concentration.*

Test–tank	Exposure concentration, mg/L SCN^-	Number of fish	Percent mortality at 96 h
651–6	518	10	90
651–5	375	10	90
651–4	298	10	80
651–3	232	10	80
652–6	198	10	90
651–2	163	10	80
652–5	148	10	70
652–4	115	10	90
653–6	96.4	15	73
652–3	90.8	10	90
653–5	72.1	15	47
652–2	65.4	9	56
653–4	56.1	15	73
653–3	44.3	15	53
653–2	31.6	15	47
655–6	31.3	10	50
655–5	24.1	10	70
655–4	18.5	10	30
655–3	14.8	10	20
655–2	10.5	10	0
All tests–1	0.0	45	0

[a] Mean values and ranges for all tanks were: fish size, 6.7 g (5.6–7.9); water temperature, 15.1°C (12.9–17.3); dissolved oxygen, 7.7 mg/L (6.4–8.3); pH, 7.88 (7.83–7.90); alkalinity, 176 mg/L as $CaCO_3$ (167–181); and hardness, 203 mg/L as $CaCO_3$ (195–208).

TABLE 3.—*Percent mortality before and after stress of trout exposed to thiocyanate. Fish were stressed (chased with a dip net) for 5–15 s after 96 h of exposure; mortality was measured either 0.25 or 1.25 h later.*

	Brook trout[a], Test 658			Rainbow trout[b], Test 723		
	Exposure concentration,	Percent mortality		Exposure concentration,	Percent mortality	
Tank	mg/L	96 h	96.25 h	mg/L	96 h	97.25 h
1	0	0	0	0	0	0
2	5.5	0	10	7.7	0	100
3	8.0	10	70	9.9	0	100
4	10.0	10	70	11.8	30	80
5	13.0	0	80	15.3	0	100
6	16.7	10	100	20.8	50	90

[a] 96-h LC50 (median lethal concentration) > 16.7 mg/L; 96.25-h LC50 = 7.8 mg/L (95% confidence interval, 6.6–9.2). Mean fish size, 8.9 g. Mean values and ranges for water variables in all tanks: temperature, 16.3°C (15.9–16.7); dissolved oxygen, 7.5 mg/L (7.2–7.9); pH, 7.86 (7.83–7.95); alkalinity, 164 mg/L as $CaCO_3$ (all measurements); hardness, 194 mg/L as $CaCO_3$ (194–195).

[b] 96-h LC50 = 20.8 mg/L (95% confidence interval not calculable by method used); 97.25-h LC50 < 7.7 mg/L. Mean fish size, 0.7 g. Mean values and ranges for water variables in all tanks: temperature, 11.8°C (11.3–12.5); dissolved oxygen, 9.4 mg/L (9.2–9.8); pH, 7.95 (7.92–8.00); alkalinity, 175 mg/L as $CaCO_3$ (172–178); hardness, 202 mg/L as $CaCO_3$ (198–206).

rainbow trout at different time intervals (0–48 h) after toxicant addition to the incoming water was discontinued. The syndrome persisted, but with decreasing severity and frequency, for up to 24 h after toxicant addition ended (Table 5).

Physiological Effects

Groups of 20 fish each were exposed for 3–5 d to concentrations between 0 and 23.2 mg/L SCN^- to examine the relationship between plasma thiocyanate concentration and SDS. After the exposure periods, there were 16–20 survivors in each group, of which 10 fish were randomly selected and stressed (dip-net method), and the occurrence of SDS was recorded; plasma thiocyanate concentrations were measured in the balance of each group. There was an excellent dose–response correlation between measured plasma thiocyanate concentrations and occurrence of SDS in the corresponding subgroups of stressed fish (Fig. 1); the calculated median response (50% SDS mortality) occurred at 246 mg/L SCN^-.

Rainbow trout took up and accumulated significant amounts of SCN^- when exposed to thiocyanate (Table 6). This was accompanied by a decrease in plasma chloride, but had no effect on plasma pH or total CO_2 content (Table 6). The thiocyanate concentration of plasma equaled that of the test water within 24 h, after which uptake of SCN^- from the test water continued against its concentration gradient (Table 6). The rate at which thiocyanate accumulated in the plasma was a function of exposure concentration and of fish size (Fig. 2). The accumulation rate increased when exposure concentration increased but demonstrated saturation kinetics above approximately 29.0 mg/L SCN^-. At any given exposure concentration, smaller fish accumulated thiocyanate more rapidly than larger fish.

Uptake of thiocyanate by rainbow trout was inhibited by the presence of equimolar quantities of other univalent anions in the exposure water, in the following order of inhibitory effect: $Cl^- > Br^- > I^- > F^-$ (Table 7). Acetate ($C_2H_3O_2^-$) was without significant effect on thiocyanate uptake.

The rate at which thiocyanate was excreted in urine was proportional to the plasma concentration of thiocyanate (Fig. 3), but was extremely slow relative to the rate of plasma accumulation

TABLE 4.—*Acute toxicity of thiocyanate to rainbow trout during tests with different light regimes.*[a] *After 96 h of exposure to the toxicant, fish in continuous red light were subjected to a brief pulse of white light and those in continuous white light were subjected briefly to total darkness; mortality was measured 0.75 h later. Median lethal concentration = LC50; confidence interval = CI.*

Test	Light regime	96-h LC50, mg/L SCN^- (95% CI)	96.75-h LC50, mg/L SCN^- (95% CI)
721	Continuous red light	159 (119–213)	76.3 (48.7–120)
725	Continuous red light	64.6 (13.2–316)	64.6 (13.2–316)
727	12 h white light : 12 h dark	83.5 (not calculable)	
728	12 h white light : 12 h dark	55.7 (39.0–79.6)	
720	Continuous white light	48.3 (34.6–67.5)	38.7 (25.7–58.3)
724	Continuous white light	44.9 (30.0–67.2)	41.4 (27.4–62.5)

[a] Mean values and ranges for all tanks: fish size, 0.8 g (0.3–1.8); water temperature, 13.0°C (12.2–13.5); dissolved oxygen, 9.0 mg/L (8.7–9.3); pH, 7.93 (7.77–8.87); alkalinity, 174 mg/L as $CaCO_3$ (168–206); hardness, 202 mg/L as $CaCO_3$ (200–206).

(Fig. 2). Exposure to 35 mg/L SCN^- for 96 h did not significantly alter the urine flow rate (mean rate: pre-exposure 97.4 ± 18.5 mL/d·kg, $N = 4$; postexposure 111.9 ± 9.7 mL/d·kg, $N = 13$) or the chloride content of urine (mean concentration: pre-exposure 255.6 ± 32.0 mg/L, $N = 4$; postexposure 202.4 ± 21.3 mg/L, $N = 13$). Urinary excretion of thiocyanate was far too slow to account for the disappearance of SDS within 48 h after exposure to thiocyanate was discontinued (Table 5). This suggests that, in the absence of external toxicant, thiocyanate was also excreted across the gills.

A relatively small but highly significant portion of blood thiocyanate was converted to cyanide (Table 8). Cyanide is virtually un-ionized within the physiological pH range of fish (Izatt et al. 1962) and, consequently, blood cyanide probably was present as HCN. The endogenous conversion of thiocyanate to cyanide produced an average molar equilibrium ratio of SCN^- to HCN in blood (mean haematocrit 25%) of 664:1. Further conversion of SCN^- to form cyanate (OCN^-) and ammonia in blood (Oram and Rei-

TABLE 5.—*Persistence of sudden death syndrome (SDS) in rainbow trout (size range, 0.4–2.5 g) exposed to 7.3 mg/L SCN^- for 96 h, maintained in fresh water for 0–48 h, and then stressed with a dip net for 30 s (N = 10).*[a]

Group	Fish alive after 96-h exposure	Hours in replacement water after exposure and before stress[b]	Deaths (SDS) during 45 min after stress	Percent mortality from SDS
1	8	0	6	75
2	10	4	6	60
3	9	12	2	22
4	9	24	3	33
5	9	48	0	0

[a] A single control group of 10 fish was maintained in fresh water for the entire 96-h exposure and subsequent 48-h stress periods. This group was stressed immediately at the close of the 96-h exposure period and subsequently at 4, 12, 24, and 48 h. No mortalities occurred in this control group.

[b] No fish died during these periods.

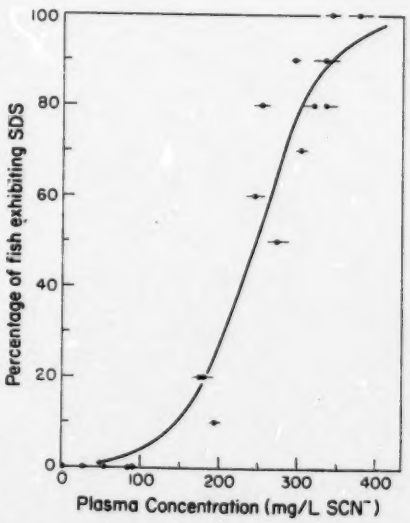

FIGURE 1.—*Prevalence (y) of sudden death syndrome (SDS) in rainbow trout exposed to KSCN at 10.8°C, in relation to plasma thiocyanate concentrations (x). Entries are means ± SE (N = 6–10) for plasma data; 10 fish (mean size ± SE, 7.0 ± 0.2 g) determined each SDS percentage. Least-squares regression line is* $y = 1/[1 + \exp(5.460 - 74.807x)]$; $r = 0.882$. *Median (50%) response occurred at 246.3 mg/L SCN^-.*

TABLE 6.—*Ionic and acid–base status of blood of cannulated rainbow trout (mean size ± SE, 168 ± 9 g) exposed to KSCN at 6.9°C. Entries are means ± SE (N = 6).*

Exposure time, h	Haematocrit, %	Plasma pH	Total plasma CO_2, mg/L	Plasma SCN^-, mg/L	Plasma Cl^- g/L
			Unexposed fish (water SCN^-, 0.0 mg/L)		
0	22 ± 2	7.91 ± 0.05	440 ± 70	0.3 ± 0.4	4.06 ± 0.10
24	17 ± 2	7.90 ± 0.05	475 ± 48	0.5 ± 0.5	4.11 ± 0.11
48	14 ± 2	7.91 ± 0.05	519 ± 44	1.0 ± 0.8	4.02 ± 0.08
96	11 ± 2	7.89 ± 0.03	462 ± 44	1.0 ± 0.6	4.00 ± 0.11
			Exposed fish (water SCN^-, 8.7 ± 0.4 mg/L)		
0	21 ± 2	7.93 ± 0.04	532 ± 48	0.5 ± 0.5	4.13 ± 0.17
24	14 ± 2	7.92 ± 0.04	515 ± 31	10.7 ± 1.0[a]	4.17 ± 0.19
48	13 ± 5	7.92 ± 0.02	563 ± 48	21.9 ± 2.3[a]	3.78 ± 0.15[a]
96	8 ± 2	7.94 ± 0.02	515 ± 22	40.6 ± 2.3[a]	3.49 ± 0.11[a]

[a] Different from time-zero value at $P \leq 0.05$.

ter 1966; Chung and Wood 1971) was not detected. Blood NH_3-N concentrations were not found to change significantly when blood SCN^- concentrations increased nearly two orders of magnitude (Table 8).

Discussion

Thiocyanate competed successfully with other univalent anions, notably chloride, for uptake across the gills of freshwater trout and, in this way, SCN^- was accumulated in the body against its concentration gradient. This situation is the reverse of that which occurs in seawater fish; in seawater fish, thiocyanate competes with chloride for excretion across the gills and plasma thiocyanate levels remain below those of the seawater (Epstein et al. 1973; Epstein et al. 1975). The mechanism responsible for thiocyanate movements in both instances is presumed to be substitution of SCN^- for Cl^- at the HCO_3^-/Cl^- exchange sites of the gills. Although thiocyanate inhibited chloride uptake in fresh water, it had no effect on urinary loss of plasma chloride. The net result was a decrease in plasma chloride during thiocyanate exposure. Uptake of thiocyanate was inhibited not only by external Cl^- but also by external Br^-, I^-, and F^-. Hence, the presence of these anions in the external environment would be expected to decrease the relative toxicity of thiocyanate. An extrapolation of this anionic effect leads to the prediction that thiocyanate is

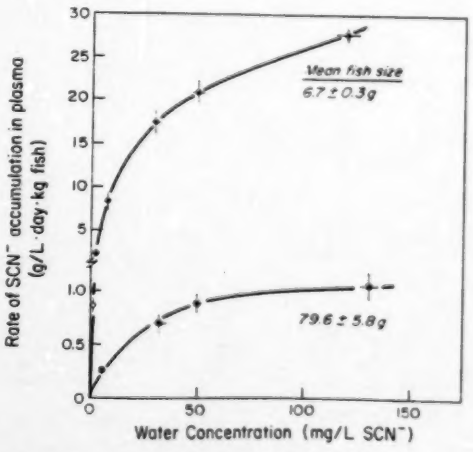

FIGURE 2.—*Accumulation rate (y) of thiocyanate in plasma of rainbow trout exposed to KSCN for 96 h at 8.4°C, showing effects of exposure concentration (x) and fish size. Entries are means ± SE (N = 6–11). Least-squares regression lines: for 6.7-g group, $y = 390.7 + 103.9 \log_{10} x$, $r = 0.991$; for 79.6-g group, $y = 19.4 + 5.2 \log_{10} x$, $r = 0.998$.*

TABLE 7.—*Average rate of thiocyanate accumulation in plasma of rainbow trout (mean size ± SE, 74 ± 5 g) during 96 h of exposure at 8.4°C to 47.6 mg/L SCN^- and an equimolar concentration of KCl, KBr, KI, KF, or $NaC_2H_3O_2$. Entries are means ± SE (N = 5–7).*

Anions present in exposure water	Rate of accumulation, mg/(L·d·kg)	Percent SCN^- accumulation compared to control fish
SCN^- (control)	865 ± 87	
SCN^- and $C_2H_3O_2^-$	813 ± 52	94
SCN^- and F^-	680 ± 93	79
SCN^- and I^-	430 ± 93[a]	50
SCN^- and Br^-	331 ± 23[a]	38
SCN^- and Cl^-	209 ± 12[a]	24

[a] Different from control value at $P \leq 0.05$.

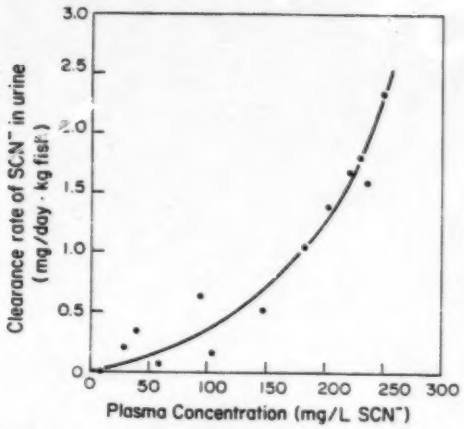

FIGURE 3.—*Excretion rate of thiocyanate in urine of rainbow trout exposed to 34.8 mg/L SCN⁻ for 96 h at 12.3°C, in relation to the plasma SCN⁻ concentration. Each point represents datum from one fish (mean size ± SE, 164 ± 16 g; N = 4).*

TABLE 8.—*Relationship between thiocyanate, cyanide, and total ammonia-nitrogen concentrations in blood of cannulated rainbow trout (mean size ± SE, 204 ± 38 g) exposed to 51.4 mg/L SCN⁻ at 12.7°C. Entries are means ± SE (N = 4).*

Exposure time, h	SCN^-, mg/L	CN^-, µg/L	NH_3-N, mg/L
0	1.1 ± 0.5	3.9 ± 0.03	2.3 ± 0.2
48	37.2 ± 5.1[a]	25.2 ± 0.08[a]	2.4 ± 0.1
96	71.4 ± 4.1[a]	32.0 ± 0.03[a]	1.6 ± 0.5

[a] Different from time-zero value at $P \leq 0.05$.

less toxic in seawater than in fresh water. This does not appear to have been the case, however, in a test conducted by Doyle (1977) on guppies *Lebistes reticulatis*; survival time of guppies exposed to 20 mmol/L NaSCN (1,162 mg/L SCN^-) in seawater was half that of guppies in fresh water. This may be related to general impairment of the ionic and osmotic regulatory capabilities of the gill; thiocyanate may have directly affected the ability of guppies to withstand the transfer to seawater.

Thiocyanate exposure had no effect on plasma pH or total CO_2. This was unexpected inasmuch as thiocyanate inhibits the activity of carbonic anhydrase (Davenport 1940), the enzyme responsible for catalysing the interconversion of CO_2 and HCO_3^- in gill tissue and red blood cells. Carbonic anhydrase plays a determinant role in the exchange of ions and gas (CO_2) across the gill (for review see Maren 1967); abolition of carbonic anhydrase activity induces an internal acidosis and an elevation in total body CO_2 stores.

The rate of thiocyanate uptake, and presumably the relative toxicity of thiocyanate, depended on fish size; this is to be expected because of the higher metabolic rate and therefore the higher gill transfer rates of smaller fish. One would expect other factors that alter gill transfer rates, including water temperature, ambient calcium concentrations or hardness, and catecholamine release (Pickering 1981; Houlihan et al. 1982), also to affect the uptake and relative toxicity of thiocyanate.

Several studies have documented the endogenous conversion of thiocyanate to cyanide (Goldstein and Reiders 1951, 1953; Boxer and Rickards 1952b; Pines and Crymble 1952). The molar equilibrium ratio of SCN^- to HCN in body fluids following injection of thiocyanate salts is approximately 1,000:1 in mammals (Goldstein and Reiders 1951; Boxer and Rickards 1952b). Our value for the equilibrium ratio in fish blood is in general agreement with the mammalian data. All of these aforementioned studies, as well as the present one, used an acidification method to separate thiocyanate from cyanide. The validity of such separation techniques when used with blood has been questioned by Vesey and Wilson (1978), who demonstrated that acidification of thiocyanate solutions containing haemoglobin resulted in artifactual formation of cyanide. They concluded that the activity of haemoglobin in the conversion of SCN^- to HCN, as reported earlier by Chung and Wood (1971), is of little physiological importance. They also concluded that evidence for the red cell enzyme "thiocyanate oxidase," as reported by Goldstein and Reiders (1953), is artifactual in nature. The results of Vesey and Wilson (1978), however, do not account for cyanide found in respiratory gas (Boxer and Rickards 1952a) or in haemoglobin-free plasma (Goldstein and Reiders 1951) after administration of thiocyanate. It follows that endogenous conversion of SCN^- to HCN is not precluded. It is not known if the SCN^-:HCN equilibrium ratio in fish blood is shifted during stress or if endogenously generated cyanide is involved in SDS.

The present results demonstrate that thiocy-

anate is acutely toxic to trout at concentrations lower than those suggested by previous studies (e.g., Doudoroff 1976). The acute toxicity of thiocyanate to trout, however, is relatively unpredictable. Exposed fish that seemed healthy would suddenly, and often for no apparent reason, go into convulsions and quickly expire. The stimulus of sudden, rapid movement this provided often was enough to trigger SDS in neighbouring fish. The signs of SDS in trout (convulsions, loss of equilibrium and buoyancy, extreme rigor, among others) are virtually identical to the characteristics of acute thiocyanate toxicity in mammals, including humans (convulsions, vertigo, coma, hyper-reactivity, extension rigidity: Garvin 1939; Smith 1973). In mammals, these effects are probably due to the SCN^- ion itself, rather than to endogenously generated HCN; administration of sodium thiosulphate, an antidote for cyanide poisoning, has no effect on thiocyanide toxicity in mammals (Smith 1973). Sudden death syndrome in fish exposed to thiocyanate may represent a direct effect of the thiocyanate anion on neuromuscular functioning. Thiocyanate toxicity in mammals appears to be influenced by neural activity, inasmuch as sympathomimetic stimulants (e.g., amphetamines) increase the toxicity of SCN^-, whereas barbiturate anaesthetics (e.g., phenobarbital) decrease its toxicity (Smith 1973). Moreover, SCN^- enhances the contractility and electrical conduction of frog muscle fibres (Lubin 1957), and increases the excitability and electrical transmission of spinal neural pathways and motoneurons (Goto and Esplin 1961). Regardless of the exact mechanism involved in SDS, normal rapid movements of wild fish during feeding and predator avoidance probably would be sufficient to trigger SDS as a consequence of thiocyanate exposure.

Acute toxicity tests (96-h LC50 determinations) were of limited value in predicting the toxicity of thiocyanate because of the unpredictability of SDS. A much better understanding of the relationship between stress, thiocyanate exposure, and SDS is required for LC50 determinations to be meaningful. For this reason, we have presented our actual mortality data and have avoided calculation of LC50 values where possible. The correlation between plasma thiocyanate concentrations and SDS provided a more certain measure of thiocyanate toxicity. The results demonstrate that, under our test conditions, 50% of the fish would be at risk of SDS when plasma SCN^- concentrations reached approximately 250 mg/L. The duration of thiocyanate exposure necessary to attain such a concentration depended upon exposure concentration, fish size, and ambient water quality. It is possible that, under conditions when uptake of SCN^- is extremely slow, excretion of SCN^- would be able to keep pace with SCN^- uptake, and plasma concentrations of thiocyanate would remain low. Under such circumstances, SDS would not be expected to occur. None of our experiments was run at a sufficiently low concentration of thiocyanate to test this hypothesis; the lowest concentration of toxicant we tested was 1.0 mg/L SCN^-. At that level of exposure, 6.7-g rainbow trout accumulated thiocyanate at a rate of approximately 870 mg/(L plasma·day·kg). Given such a rate of SCN^- accumulation, one would expect that 50% of the exposed population would be at risk of SDS within 1–2 months. During such long-term exposures, fish probably would encounter the chronic toxic effects of thiocyanate.

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